Journal of Chromatography, 167 (1978) 41–65
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CHROM. 11,027

THE ROLE OF THE TEMPERATURE IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING PYROCARBON-CONTAINING ADSORBENTS

HENRI COLIN, JOSÉ CARLOS DIEZ-MASA and GEORGES GUIOCHON

Laboratoire de Chimie Analytique Physique, École Polytechnique, Route de Saclay, 91128 Palaiseau Cedex (France)

and

TERESA CZAJKOWSKA and IRÉNA MIEDZIAK

Department of Physical Chemistry, Marie Curie Sklodowska University, Nowotki 12, Lublin (Poland)

SUMMARY

The investigation of the effect of temperature in liquid chromatography on pyrocarbon-containing adsorbents is considered in terms of heats of transfer, solvent eluotropic strength, column linear capacities, pressure drops and efficiencies. The selectivity of the system is scarcely altered on changing the temperature; it decreases by less than 10% when the temperature increases from ambient to 65 °C. The heat of transfer, which is determined by the nature of the mobile phase, increases directly with increasing capacity factor of the solute. The eluotropic strength of the solvent is hardly affected by the temperature. The linear capacity of the column increases two-fold with a 40 °C increase in temperature. The greater effect of temperature on the column efficiency and pressure drop is due to the decrease in the solvent viscosity, resulting in larger diffusion coefficients of the solutes.

INTRODUCTION

Whereas temperature is one of the most important parameters in gas chromatography (GC), it appears to be the one most often neglected in modern liquid chromatography (LC). One of the two major reasons for this is that most mobile phases currently used in LC have boiling points lower than 100 °C and consequently the range of temperature available is practically limited to 15–100 °C. On the other hand, very important changes in the capacity factors of solutes can be realized by altering the composition of the mobile phase, and in that respect the eluent in LC has a role comparable to temperature in GC.

It should be noted that even though temperature is an important parameter in LC, few workers have investigated its implications. Knox and Vasvari[1], working with Permaphase ETH and ODS, have shown that an increase in the temperature of the column results in better efficiencies owing to a decrease in the solvent viscosity. Thus the coefficients of the Knox reduced HETP (h) equation (h *versus* the reduced solvent

velocity, v) are independent of the temperature. Particularly from the lack of dependence of the coefficient C on temperature, they concluded that the activation energy for the rate-determining process in equilibration is close to that for diffusion in the mobile phase. These workers also measured the heats of transfer (ΔH) for different solutes and could determine whether or not retention was controlled by ΔH. Schmit *et al.*[2] and Kirkland[3] also measured HETP curves at various temperatures and similarly concluded that the increase in efficiency was due solely to the decrease in solvent viscosity. The former group also showed that the linear capacity of a column increased 2- or 3-fold on a temperature change of 60 °C. Kikta and Grushka[4], working with Corasil II bonded with n-C_{18} moieties, observed that the peak asymmetry was independent of the column temperature and that the larger the solute capacity factor (k'), the larger was the value of ΔH. However, in another study[5], the same workers, using a different packing material, found that the peak asymmetry decreases with increasing temperature (linearization of the partition isotherm) and that the increase in column efficiency could also be due to a change in the kinetic processes which occur in the system. Other studies have also dealt with temperature effects[6–8], including for instance a report by Majors[9] that temperature has only a slight influence on k', specifically that an increase of 35 °C about halves the values of k'. The author's opinion was that 50 °C is the optimal column temperature.

In previous studies on the use of pyrocarbon supports for high-performance liquid chromatography (HPLC)[10–12], disadvantages sometimes associated with these packings have been mentioned such as the low linear capacity (θ) of the column, the peak asymmetry and the very strong adsorption that occurs with some compounds. This study presents the results of an investigation on the role of temperature on some of the most important characteristics of a pyrocarbon chromatographic system: column efficiency and linear capacity, pressure drop, heats of transfer, solvent eluotropic strength and selectivity. As exemplified below, it is not easy to describe in accurate terms the contribution of temperature to given parameters, whether they relate to a change in the solute capacity factor, when the same solvent is used, or to a change in the eluent composition, when k' is kept constant. The possibility of temperature programming, as recently reported by Kikta *et al.*[13], has not been investigated in this work and deserves further attention.

EXPERIMENTAL

Various liquid chromatographic equipment was used. The pumping system included a Waters Model 6000 A (Waters Assoc., Milford, Mass., U.S.A.) and a Tracor Constametric 990 pump (Tracor Instruments, Austin, Texas, U.S.A.). The detectors were fixed-wavelength photometers operating at 254 nm: Waters Model 440, Tracor Model 960 and Spectra Physics Model 8200 (Spectra Physics, Santa Clara, Calif., U.S.A.). Injections were made using either home-made septum-type injector ports or a Rheodyne 7120 sampling system. The thermostatic units were Haake RV 20 (Haake, Berlin, G.F.R.). Columns were made with $\frac{1}{8}$-in. O.D. stainless-steel tubing (2.17 mm I.D.), and were placed inside a thermostated ethylene glycol bath. Because of their various lengths, never shorter than 60 cm, it was found more convenient to have them coiled. The columns were coiled prior to packing using the balanced-density high-pressure method.

The behaviour of various packing materials was investigated. They are listed in Table I together with the characteristics of the columns. Two kinds of pure carbon (Black Pearls L and Sterling FT.FF) were selected for this study as well as different types of silica coated with pyrocarbon: Spherosil XOB 75 and XOB 30 (Rhone-Poulenc, France). These adsorbents were prepared by the technique previously described[11,14].

TABLE I

CHARACTERISTICS OF THE COLUMNS USED

Column No.	*Packing material*	*Particle size (μm)*	*Column dimensions*	
			Length (cm)	*Diameter (mm)*
1	Spherosil XOB 75, 10.2% pyrocarbon	20	40	2.17
2	Black Pearls L	30	60	2.17
3	Spherosil XOB 75, 22.0% pyrocarbon	40	62	2.17
4	Spherosil XOB 30, 1.5% pyrocarbon	40	60	2.17
5	Spherosil XOB 30, 38% pyrocarbon	40	60	2.17
6	Spherosil XOB 30, 7% pyrocarbon	40	60	2.17
7	Sterling FT.FF	30	80	2.17
8	Spherosil XOB 75, 4.2% pyrocarbon	250	230	2.17

When necessary, chromatograms were recorded with a data system comprising a modular Solarton numerical voltmeter and a home-made voltmeter–computer interface.

Water was doubly distilled and the organic solvents were of analytical-reagent grade from Merck (Darmstadt, G.F.R.). Solute samples were of various origins. The compositions of solvent mixtures are always given by volume at 20 °C.

RESULTS AND DISCUSSION

These investigations were undertaken to study two fundamental classes of properties of liquid chromatographic systems, namely the thermodynamic properties, which include heat of transfer, column linear capacity, solvent strength and selectivity, and the hydrodynamic and kinetic properties, such as pressure drop and column efficiency.

Thermodynamic properties

Heats of transfer. The capacity factors are acutely sensitive to the retention time, t_0, of an unretained solute. Problems encountered in the determination of the column dead volume have been reported previously[12,15]. The solvents used in that study were mixtures of water (W) and methanol (MeOH) or water and acetonitrile (MeCN). Injections of pure W, MeOH or MeCN in such systems frequently exhibit

complex perturbation signals with positive and negative peaks probably caused by changes in the refractive index. Apparently, when the water content is not close to 100%, W, MeOH and MeCN are non-sorbed entities particularly useful in measuring the retention time, t_0. Because of the special optical design of the cell of the Waters Model 440 UV detector, these perturbations appear to have been eliminated and instead single, well defined peaks are obtained. In most instances, the retention time for W (negative signal), MeOH and MeCN (positive signals) are identical. No accurate measurement has been made in our laboratory in the W concentration ranges 0–5% or 95–100%, but the phenomenon reported by Scott and Kucera[16] is not believed to occur in our systems.

When the column temperature is higher than that of the pump, the volume occupied within the column by a given amount (mass) of solvent is larger than that of the same amount at the outlet of the pump: this is naturally caused by thermal expansion of the solvent. The density of a liquid (d) decreases with increasing temperature (T°K), and this phenomenon is described by a polynomial equation of the temperature. Densities of W–MeOH and W–MeCN mixtures were measured at various compositions and temperatures in order to explain changes in the values of t_0 observed at increasing temperature. In the range 15–65 °C, it is possible to fit the experimental points of d *versus* T on a straight line with an excellent coefficient of correlation ($\geq$ 0.995). The relationship between density and temperature is given by the following equation:

$$d = d_0[1 - k_e(T - 273)] \quad (1)$$

where d_0 is the density at 0 °C and k_e the coefficient of expansion. Some results are given in Table II. It should be noted that d_0 has no physical significance and is used here simply as a constant to solve the equation for the fit within the range 15–65 °C.

The ratio of retention times, t_0, at temperatures T_1 and T_2 is easily obtained by

$$\frac{t_0^{T_1}}{t_0^{T_2}} = \frac{1 - k_e(T_1 - 273)}{1 - k_e(T_2 - 273)} \quad (2)$$

Other contributions may also alter the value of t_0. For instance, the stainless-steel tubing of the column also expands, resulting in an increase in t_0. In addition, at higher

TABLE II

THERMAL EXPANSION COEFFICIENTS (k_e) OF METHANOL–WATER AND ACETONITRILE–WATER MIXTURES

The compositions are given in % (v/v) of water at 20.5°C.

Parameter	*Water content (%, v/v)*										
	0	*10*	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*	*100*
k_e(MeCN) ($\times 10^3$)	1.3547	1.2525	1.1871	1.1112	1.0188	0.9245	0.8320	0.7430	0.6638	0.5676	0.3869
d_0(MeCN)	0.8098	0.8308	0.8559	0.8808	0.9040	0.9264	0.9475	0.9669	0.9850	1.000	1.007
k_e(MeOH) ($\times 10^3$)	1.1767	1.0577	0.9858	0.9219	0.8509	0.7754	0.6924	0.6038	0.5236	0.4547	0.3869
d_0(MeOH)	0.8103	0.8450	0.8743	0.9000	0.9226	0.9416	0.9577	0.9714	0.9829	0.9938	1.007

temperature a larger part of the porous structure of the packing material may become more accessible to the mobile phase because of its decreased viscosity. However, these contributions are expected to be of minor importance as in many instances the change in t_0 observed experimentally was in good agreement with that predicted by eqn. 2 (within 5–10%).

The dependence of the capacity factor, k', on temperature is given by

$$\ln k' = \frac{\Delta H}{RT} - \frac{\Delta S^\circ}{R} + \ln \frac{A}{V_M} \tag{3}$$

where ΔH is th enthalpy of transfer from the stationary to the mobile phase, ΔS° is the associated change in standard entropy (standard state is unit molar concentration in each phase) and A is the total surface area of the adsorbent in the column of dead volume V_M. Assuming that ΔH, ΔS° and A/V_M are independent of temperature, eqn. 3 suggests the classical dependence between $\ln k'$ and $1/T$.

In one series of experiments, the values of ΔH and ΔS° were calculated for several solutes, mainly homologous non-polar substances. If calculation of A/V_M is easy for pure carbon adsorbents (BET measurements for A), it is more difficult for pyrocarbon-modified silica gel. Indeed, in some instances the fraction of silica surface covered by the film of pyrocarbon can be notably different from 100%. However, as was previously done[17], values of A for such packings have been evaluated by comparison of retention data.

The results are given in Table III. The ΔH values are not very large, although they are larger than those given in the literature for other reversed phase systems (see, for instance, ref. 1). As will be seen in a subsequent section, the larger the solvent water content, the larger is ΔH. With pure MeOH, ΔH is typically in the range 2.5–5 kcal/mole. In fact, the general trend is an increase in ΔH with increasing k'. This correlation is shown in Fig. 1 for the different systems studied. This observation is not an absolute one and it is possible to find instances where larger k' are associated with smaller ΔH. However, in most instances, the more retained the solute, the larger is its heat of transfer. A 3.5-fold increase in k' is accompanied by an increase in ΔH of about 1 kcal/mole. The rather low values of ΔH indicate that the effect of temperature on k' is moderate: $\Delta H = 4$ kcal/mole results in a 2-fold decrease in k' for a 30 °C temperature increase.

The correlation between ΔH and k' is excellent for compounds in homologous series. The results obtained with the n-alkylbenzenes are shown in Fig. 2 (n is the number of carbon atoms in the linear branched chain) for different systems. Such results are very similar to those obtained in GC[18] and are characteristic of adsorption processes. Working with Corasil II modified by n-C_{18} moieties, Kikta and Grushka[4] observed a surprising result for the curves of ΔH *versus* n in a homologous series of phenones, which they explained in terms of an entropy effect. With our systems, ΔS increases linearly with ΔH (and consequently with k' and n) in homologous series (Fig. 3). Similar results have been obtained in GC using graphitized carbon black[18]. The ΔS° values determined on pyrocarbon packings are much larger than those given in the literature (*e.g.*, refs. 1 and 4). Such results can probably be explained by the much stronger orientation of the solute molecules on carbon adsorbents than on

TABEL III

ENTHALPIES (ΔH) AND ENTROPIES (ΔS°) OF TRANSFER FROM STATIONARY TO MOBILE PHASE FOR VARIOUS SYSTEMS

ΔH in kcal/mole; ΔS in cal/mole · °K. For column numbers, see Table I.

Column No.	*Parameter*	*System**										
		nc_4Ph	*nc_5Ph*	*nc_6Ph*	*nc_8Ph*	*nc_9Ph*	*1,2,3-TMB*	*1,2,4-TMB*	*1,3,5-TMB*	*1,2,4-TClB*	*3,4,5-TMB*	*Picril chloride*
1	ΔH	2.203	2.710	3.116	4.451	5.023	2.640	2.855	2.586	3.729	2.602	3.668
	ΔS°	15.38	15.98	16.41	18.80	19.63	15.15	16.32	16.09	16.68	14.63	15.83
2	ΔH	—	2.559	3.010	4.052	4.710	3.004	2.925	2.897	2.835	4.065	3.675
	ΔS°	—	15.48	15.91	17.11	18.16	15.28	15.43	16.08	13.72	17.27	15.28
3	ΔH	1.348	—	2.378	3.531	4.315	2.231	2.326	—	2.998	1.171	3.499
	ΔS°	13.34	—	14.78	16.49	18.06	14.62	15.59	—	15.30	11.19	15.94
4	ΔH	4.019	4.119	47.53	5.888	6.656	4.564	4.027	4.265	4.336	5.005	5.377
	ΔS°	18.94	17.71	18.81	19.82	21.09	17.25	17.34	18.80	16.88	19.73	17.65
5	ΔH	3.044	3.346	3.686	4.527	5 078	3.436	3.538	3.123	2.894	3.890	—
	ΔS°	17.69	17.49	17.59	18.22	18.96	16.60	17.63	17.09	14.10	17.96	—
6	ΔH	2.606	4.065	3.763	5.212	5.879	3.595	3.723	3.560	3.408	4.477	—
	ΔS°	11.98	18.39	17.95	19.00	18.66	16.12	17.16	17.37	14.69	18.71	—
7	ΔH	3.412	3.728	4.363	—	—	3.603	3.835	3.639	3.864	4.532	4.986
	ΔS°	18.01	16.27	17.30	—	—	15.43	16.54	16.48	15.32	16.46	15.14

* Ph = benzene; TMB = trimethylbenzene; TClB = trichlorobenzene; TMP = trimethylphenol.

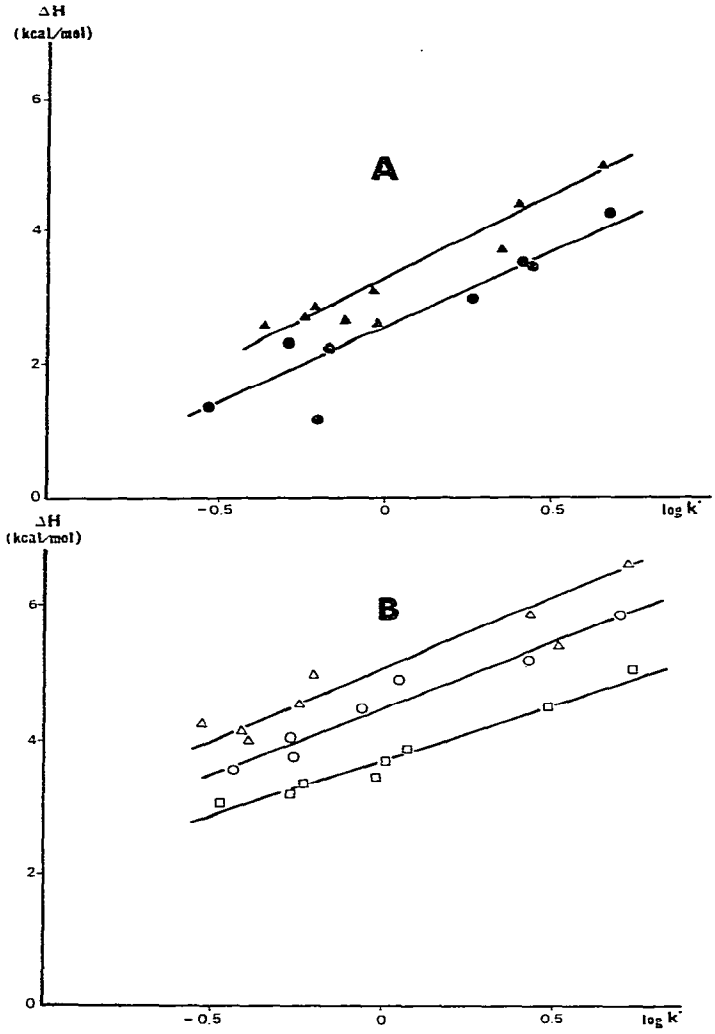

Fig. 1. Variation of the heat of transfer (ΔH, kcal/mole) with the logarithm of the capacity factor. (A) Spherosil XOB 75 + pyrocarbon; ●, column 1; ▲, column 3. (B) Spherosil XOB 30 + pyrocarbon: △, column 3; □, column 5; ○, column 6.

chemically bonded phases (especially with sample molecules containing aromatic structures). The change in the selectivity $\alpha_{i,j}(= k'_i/k'_j)$ between solutes i and j with temperature is given by

$$\frac{\alpha_{T_1}}{\alpha_{T_0}} = e^{-\frac{T_1 - T_0}{T_1 T_0} \cdot \frac{\Delta(\Delta H)}{R}} \tag{4}$$

where $\Delta(\Delta H)$ is $\Delta H_i - \Delta H_j$. As ΔH_i is generally larger than ΔH_j (solute i is more retained than solute j), the larger the temperature, the smaller is the selectivity. How-

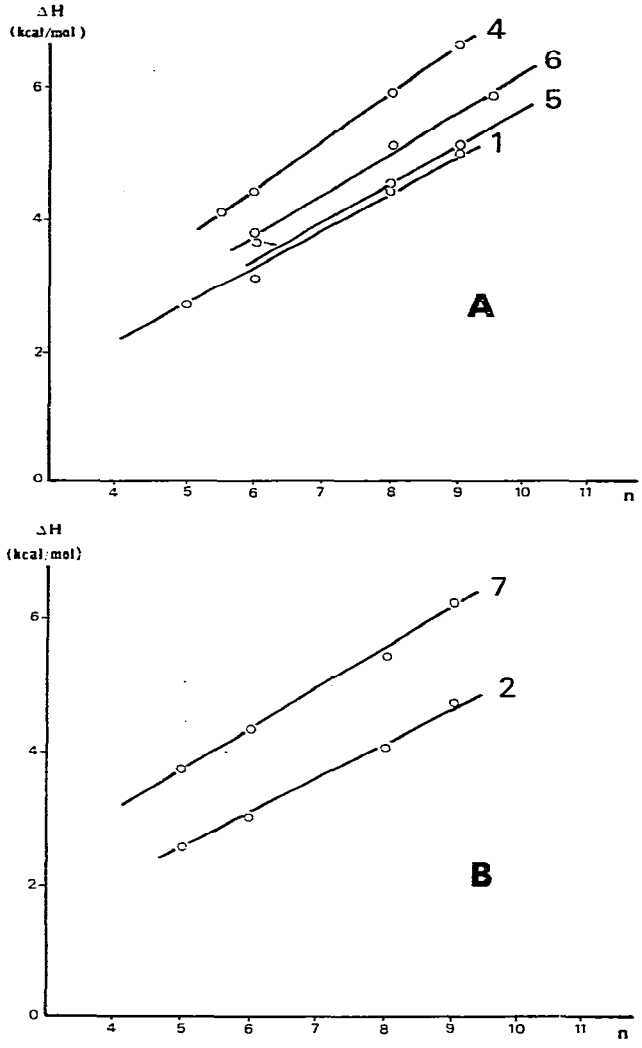

Fig. 2. Variation of the heat of transfer (ΔH, kcal/mole) with the number of carbon atoms (n) in the alkyl chain of n-alkylbenzenes. (A) Silica gel modified with pyrocarbon; (B) carbon black hardened by pyrocarbon. The numbers close to the lines refer to the columns (see Table I).

ever, if the problem is the separation of closely eluted compounds, $k'_i \approx k'_j$ and most often $\Delta(\Delta H) = 0$: α is independent of temperature.

The variation of α with temperature for two separation problems is reported in Fig. 4. The first example deals with very similar solutes, 1,2,3- and 1,2,4-trimethylbenzene, and the second one concerns the separation of n-octylbenzene and 3,4,5-trimethylphenol, that is two solutes with a large difference in polarity. The results suggest that α is fairly constant within the range of temperature studied, with a slight tendency to decrease with increasing temperature, in agreement with eqn. 4.

The influence of temperature on the selectivity has also been studied for an

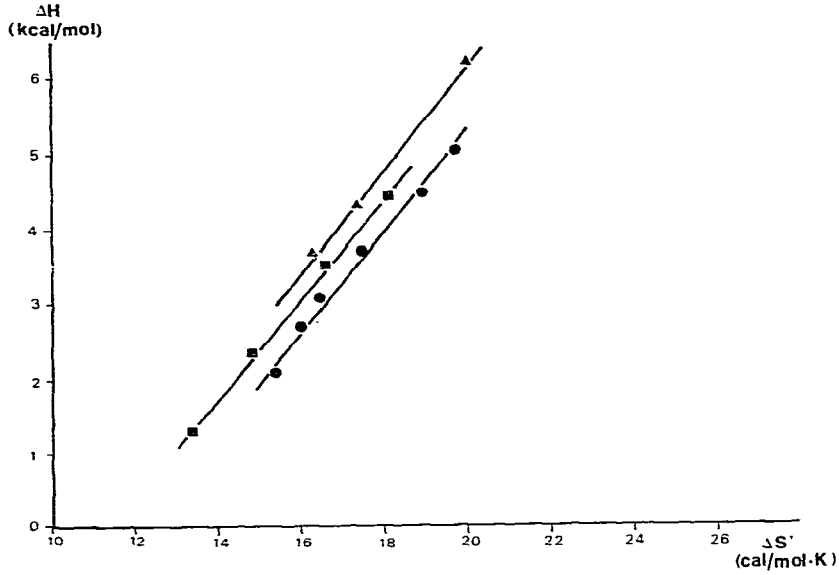

Fig. 3. Correlation between heat of transfer (ΔH, kcal/mole) and change in standard entropy ($\Delta S°$ cal/mole·°K) for homologous compounds (*n*-alkylbenzenes). Column 1; solvent, methanol.

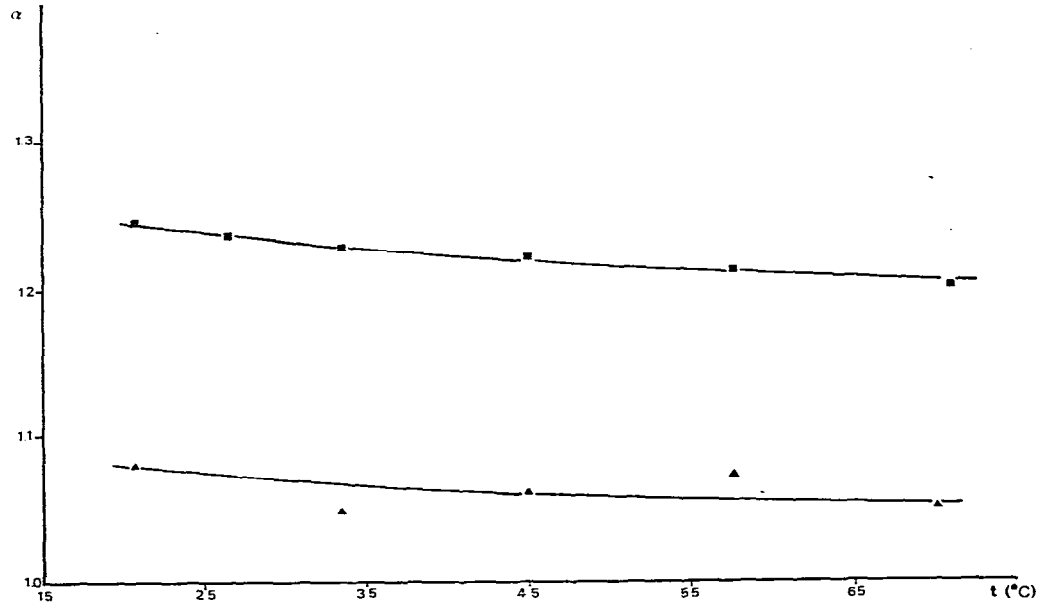

Fig. 4. Variation of the selectivity (α) with temperature (t°C). ■, Separation 1,2,3- and 1,2,4-trimethylbenzene; ▲, separation *n*-octylbenzene and 3,4,5-trimethylphenol. Column 4; solvent, methanol–16.7% (v/v) water.

actual separation problem. The variations of log k' with temperature for the various compounds studied are given in Fig. 5. It appears that the heats of transfer are very similar for all of the compounds. There is no improvement in the system selectivity or in the resolution achieved as the pairs which are difficult to separate at 20 °C cannot be resolved better at 60 °C. In some instances, increasing the temperature may even be unfavourable, as can be seen in Fig. 6. Because of slow decomposition, injections of safrole and estragole samples produce three major peaks in both instances. There is no separation problem for safrole at either 20 °C or 60 °C but it can be seen that if the last two peaks of estragole are completely resolved at room temperature, the resolution is only about 1 at 60 °C, although the efficiency is better at 60 °C than at 20 °C (see later).

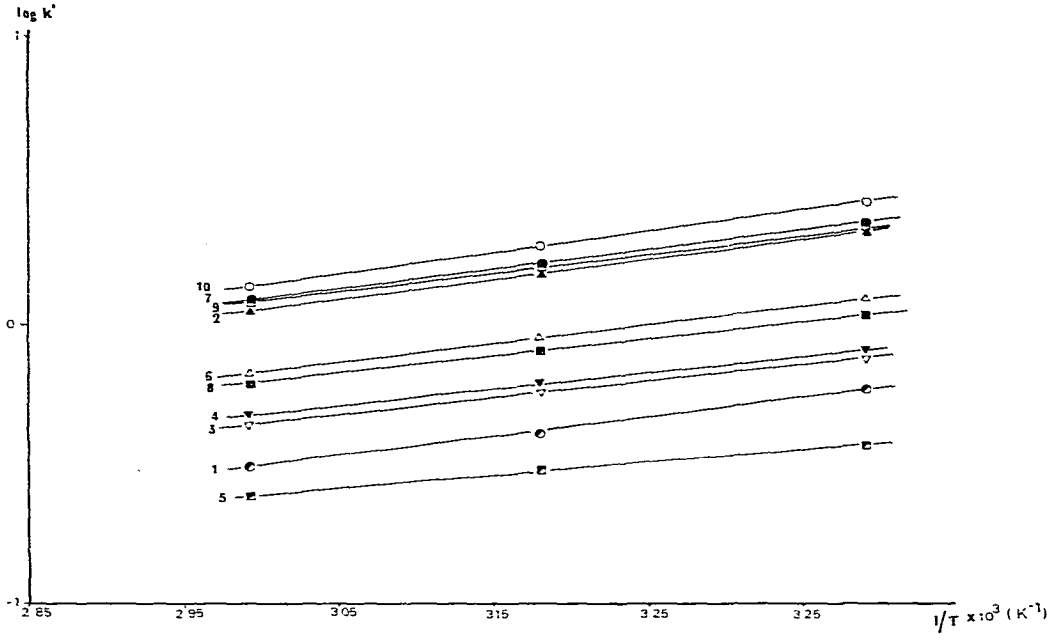

Fig. 5. Van 't Hoff plots for various compounds to be separated (see Fig. 6). Column 1; solvent, acetonitrile. 1 = Methyleugenol; 2 = cinnamyl aldehyde; 3 = cinnamyl acetate; 4 = cinnamyl alcohol; 5,6,7 = safrole; 8,9,10 = estragole.

Solvent strength (ε°). If $\varepsilon^\circ_{S_1}$ and $\varepsilon^\circ_{S_2}$ are the eluotropic strength of solvents S_1 and S_2, the capacity factors (k'_{S_1} and k'_{S_2} respectively) of a solute of molecular area a eluted by the two solvents are related through the equation given by Snyder[19]:

$$\log\left(\frac{k'_{S_1}}{k'_{S_2}}\right) = a(\varepsilon^\circ_{S_2} - \varepsilon^\circ_{S_1}) \tag{5}$$

If solvent S_1 is chosen as a reference ($\varepsilon^\circ_{S_1} = 0$), it is possible to calculate the variation of $\varepsilon^\circ_{S_2}$ with temperature:

$$\varepsilon^{\circ(T)}_{S_2} = \varepsilon^{\circ(T_0)}_{S_2} - \frac{1}{a}\left(\frac{\Delta H^{S_1} - \Delta H^{S_2}}{2.3R}\right)\left(\frac{T - T_0}{TT_0}\right) \tag{6}$$

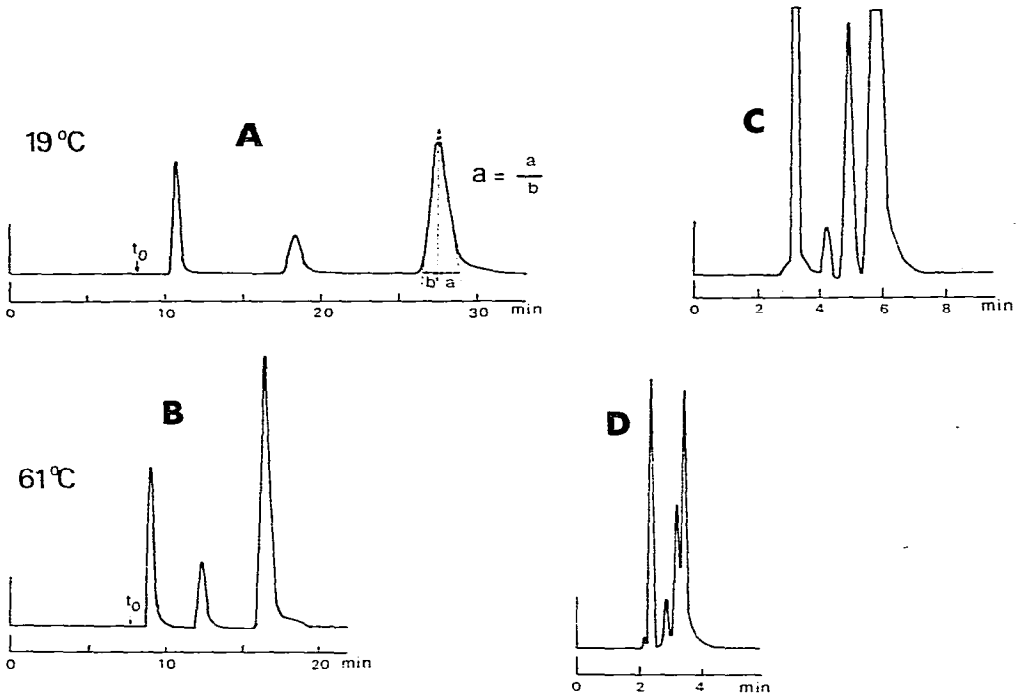

Fig. 6. Decomposition products of safrole (A and B) and estragole (C and D). Column 1; solvent, acetonitrile. Detector, UV 254 nm, 0.01 a.u.f.s. (A) temperature 19°C, flow-rate 0.3 cm³/min; (B) temperature 61.2°C, flow-rate 0.3 cm³/min; (C) temperature 19°C, flow-rate 1 cm³/min; (D) temperature 61.2°C, flow-rate 1 cm³/min. The calculation of the peak asymmetry is shown in the last peak of chromatogram A.

where ΔH^{S_i} is the heat of transfer of the solute studied from the surface to the solvent S_i. It has been observed that the smaller the solvent strength, the larger is ΔH. If $\varepsilon^\circ_{S_2}$ is positive ($k'_{S_2} < k'_{S_1}$) then $\Delta H^{S_1} > \Delta H^{S_2}$, that is $\varepsilon^\circ_{S_2}{}^{(T)} < \varepsilon^\circ_{S_2}{}^{(T_0)}$ with $T > T_0$.

The variation of the eluotropic strength of W–MeOH mixtures has been studied at different temperatures. We chose pure MeOH as a reference solvent, which means that ε° for W–MeOH mixtures is negative. Measurements of ε° were made as previously reported[10,17]. The solutes chosen were *n*-alkylbenzenes and methylphenols. Values of *a* were obtained from the data of Snyder[19]. The variations of ε° are depicted in Fig. 7. Because of the approximations made in eqn. 5 (the variations of the volume of a monolayer of solvent adsorbed on the packing as well as the contribution of activity coefficients are omitted), the ε° values calculated from the retention of alkylbenzenes and methylphenols were found to differ slightly (such results have been previously reported, in ref. 16 for instance). Eqn. 5 can only provide semi-quantitative estimates which are nevertheless useful for predicting the behavior of a system.

The data in Fig. 7 confirm that the effect of the water content of the solvent diminishes with increasing temperatures. Between 0 and 80% of water in the eluent, the decrease in ε° is proportional to the composition. The slope of the line of ε° *versus* %W decreases with temperature. The variation of the slope is linear with T and

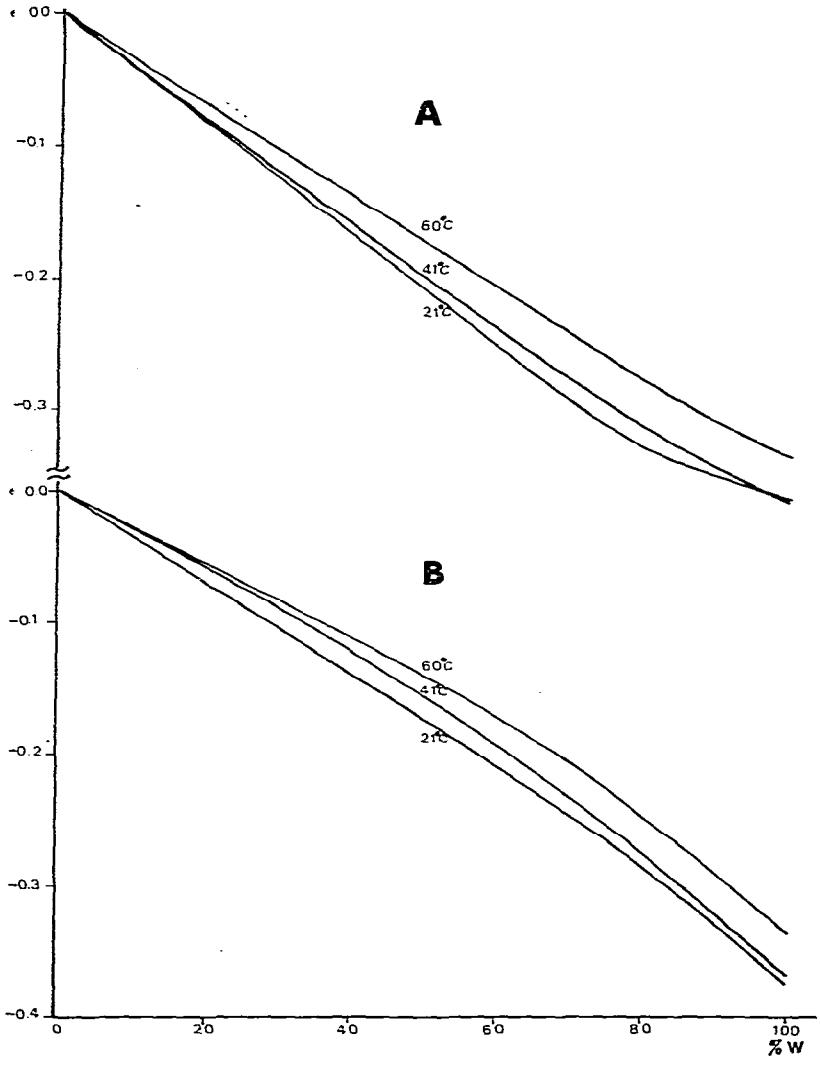

Fig. 7. Variation of the solvent strength (ε°) of water–methanol mixtures with the composition [% (v/v) water] at various temperatures (column 2). (A) ε° obtained from retention data of *n*-alkylbenzenes; (B) ε° obtained from retention data of methylphenols.

consequently the value of ε° at any composition (X%) and temperature is given by

$$\varepsilon^{\circ} = [1.90 \cdot 10^{-5} (T - 273) - 4.577 \cdot 10^{-3}]X \quad (7)$$

The relative decrease in the value of ε° is about 25% for a 50 °C increase in temperature.

Eqn. 6 suggests that $(\Delta H^{S_1} - \Delta H^{S_2})/a$ has to be constant if consistent results are to be expected. This has been tested experimentally by measurements using solutes of different polarities and molecular areas. The ensuing results shown in Fig. 8

indicate that good linearity is obtained in plotting ΔH against the water content. The figures given in parentheses are the ratio of slope to molecular area. Except with 2,3-dimethylphenol, it appears that $(\Delta H^{S_1} - \Delta H^{S_2})/a$ is independent of the solute chosen and increases linearly with the solvent water content. Such a linearity could be predicted from the proportionality between ε° and the solvent composition.

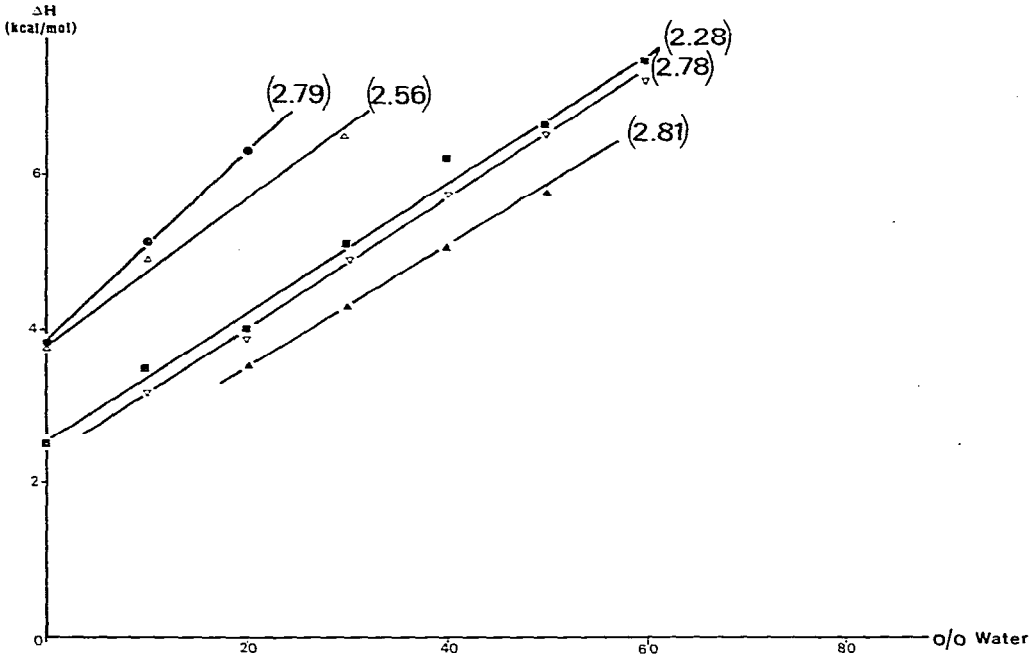

Fig. 8. Influence of the water content (%, v/v) in methanol–water mixtures on the heat of transfer (column 2). ■, 2,3-Dimethylphenol; △, 2,3,4,6-tetramethylphenol; ▲, *n*-propylbenzene; ▽, *n*-butylbenzene; ●, *n*-nonylbenzene. Figures in parentheses are the slopes of the lines divided by the solute molecular areas.

The increase in ΔH with increasing water content is responsible for the greater group selectivity associated with higher water concentrations. Variations of the selectivity, α, among homologous compounds at various temperatures and solvent compositions are given in Table IV. It then becomes evident that temperature is fairly important. An approximately 40 °C increase in the column temperature has the same effect as a 20% reduction in the water content. Should a constant selectivity be desired, as in the separation of compounds in homologous series, therefore, as the previous figures suggest, the use of higher temperatures will yield longer retention times. For instance, if α is kept constant, results obtained at 20 °C with about 10% W in the solvent will be similar to those obtained at 60 °C with 30% W. However, the capacity factor of a solute such as *n*-hexylbenzene will be three times larger in the latter instance than in the former.

As will be shown below, an increase in temperature causes an increase in column efficiency. The consequence is that working at constant selectivity will result in a larger peak capacity at high temperature (the diffusion coefficients are larger at

TABLE IV

VARIATION OF THE SELECTIVITY (α) FOR TWO COMPOUNDS OF A HOMOLOGOUS SERIES DIFFERING BY ONE CH_2 GROUP WITH THE SOLVENT COMPOSITION AND THE TEMPERATURE

Column 2 (see Table I).

Water content (%, v/v)	*Temperature (°C)*		
	20	*40*	*60*
0	1.85	1.70	1.58
10	2.00	1.84	1.76
20	2.09	2.00	1.86
30	2.28	2.11	2.00
40	2.47	2.26	2.11
50	2.56	2.44	2.23
60	3.05	2.56	2.43

60 °C in any given solvent mixture than at 20 °C in a solvent containing 20% less water).

Comparison of the results in Figs. 1 and 8 gives interesting information on the role of the packing material. In order to obtain the data given in Fig. 2, the solvent mixture has been adjusted to give the same capacity factor for a test solute (*n*-nonylbenzene) on each column. It can be seen that the smaller the pyrocarbon content of the modified silica gel, the larger the values of ΔH, which is surprising. However, the smaller the carbon content, the more polar is the solvent used. On the other hand, the data in Fig. 8 indicate that ΔH increases with the water content of the eluent. In fact, other experiments we have performed suggest that the only factor determining the heat of transfer is the chemical nature of the mobile and stationary phases. In other words, it can be said that insofar as there are not various retention mechanisms on the column, the heat of transfer is independent of the specific surface area of the packing. These results show that, in most instances, the different packings are equivalent in terms of thermodynamic properties. This is, of course, a general trend and it is likely that some solutes will exhibit a particular behaviour on a particular pyrocarbon-modified silica gel.

Column linear capacity. The fact that the linear capacity (θ) of pyrocarbon columns tends to be smaller than that of columns packed with classical material has been reported previously[12]. The value of θ is closely related to the shape of the isotherm of adsorption of the solute on the adsorbent. Snyder[19] gives three major reasons for the non-linearity of the adsorption isotherm: saturation of the solid surface by the adsorbed molecules; lateral interactions between adsorbed solute molecules; and heterogeneity of the adsorbent. Snyder reports that, for most solutes used in LC, the lateral interactions begin to give a significant contribution to the non-linearity at concentrations such that the solid surface is already saturated by solute molecules. The heterogeneity of the adsorbent is a critical parameter in determining θ. If k_i is the equilibrium constant for adsorption on site "i" which is characterized by the function $f(A_i)$, related to the interaction energy between solute and site "i", then the heterogeneity is given by

$$\log\left(\frac{k_i}{k_j}\right) = \frac{Cf(X)}{T}[f(A_i) - f(A_j)] \quad (8)$$

for a two adsorption sites model; C is a constant and $f(X)$ is a property of the solute molecule X (polarizability, dipole moment,...). For a given sample, the higher the temperature, the smaller is k_i/k_j and consequently the smaller is the heterogeneity. On the other hand, the smaller $f(A_i) - f(A_j)$ the smaller is the heterogeneity. Such a situation occurs when the characteristics of sites "i" and "j" are very close, or when the mobile phase plays a moderator role in terms of specific interactions.

In order to see if the range of concentration in which the adsorption isotherm is linear is larger at high temperatures, measurements of θ for various solutes and columns were made at different temperatures. The definition of θ given here is that amount of solute injected per gram of packing which produces a 10% variation in the adsorption thermodynamic constant, *i.e.*, a 10% variation in k'.

From a thermodynamic point of view, if increasing the temperature decreases the capacity factor and consequently the slope of the adsorption isotherm, it is possible that the linear range of this isotherm is increased or that it stays constant. This has been studied with a column operated with two different solvents. Several solutes have been used and the data are given in Table V. A 40 °C increase in temperature results in a 1.5-fold increase in θ for all of the solutes. In order to test the role of the adsorbent heterogeneity (the packing material is silica modified by pyrocarbon), two solvents have been used. In the first (MeOH) there was no water while the second contained a large amount of water (20%). The water content was chosen so as to normalize the k' value of 1,2,4-trimethylbenzene. It was hoped that, if unshielded silica areas were present on the surface of the adsorbent, these active sites would be blocked by water molecules of the solvent. Thus the term $f(A_j) - f(A_i)$ in eqn. 8 should be smaller and the heterogeneity smaller. The data in Table V show that if the k' values for 1,2,4-trimethylbenzene are the same in both solvents, there are important differences for the other solutes. Except for 3,4,5-trimethylphenol, k' values are larger with the mixture MeCN–W (20%) than with pure MeOH. The situation for the phenol can probably be explained by larger specific interactions of this polar sample with the polar mobile phase. As far as linear capacities are concerned, it appears that the values for 1,2,4-trimethylbenzene and *n*-octylbenzene are larger with the MeCN-W mixture than with pure MeOH (2-fold increase); however, there is no difference for the other two solutes. This result is surprising as an improvement in θ was expected for the trichlorobenzene and the phenol.

From a chromatographic point of view, it is probably better to compare results obtained with a normalized capacity factor, which also offers an almost constant

TABLE V

LINEAR CAPACITIES OF COLUMN 1 AT VARIOUS TEMPERATURES WITH TWO DIFFERENT SOLVENTS

Solute	*Acetonitrile + 20% water*				*Methanol*			
	k' (21.5 °C)	*Linear capacity (μg/g)*			*k'* (20 °C)	*Linear capacity (μg/g)*		
		21.5 °C	*41.25 °C*	*61.75 °C*		*20 °C*	*43 °C*	*61 °C*
1,2,4-Trimethylbenzene	1.21	100	107	164	1.17	48	51	62
1,2,4-Trichlorobenzene	4.44	42	45	57	2.38	37	39	57
n-Octylbenzene	6.70	124	148	170	3.67	33	30	53
3,4,5-Trimethylphenol	1.45	47	44	68	2.47	44	51	68

resolution, and to adjust the mobile phase composition when the temperature is changed. Under these conditions, is it valuable to increase the temperature? The data in Table VI suggest that between 20 °C and 60 °C there is approximately a 2-fold increase in θ; this is about the same increase than in the previous experiment. For each solute and at each temperature, two values of θ are given. The first was obtained when measuring k' at the peak maximum ($\theta_{Max.}$) and the second when using the retention time of the peak mass-centre ($\theta_{Mom.}$). As is predictable, $\theta_{Mom.}$ is always larger than $\theta_{Max.}$ (2–3 times). Here again, a 40 °C increase in temperature results in a 2-fold increase in $\theta_{Mom.}$. Most often, when the amount injected ($Q_{inj.}$) is small (linear part of the isotherm), there is good agreement between $k'_{Max.}$ and $k'_{Mom.}$: the peak is symmetrical. When $Q_{inj.}$ increases, the departure from linearity appears simultaneously for $k'_{Max.}$ and $k'_{Mom.}$; however, the rate of decrease is faster for $k'_{Max.}$ than for $k'_{Mom.}$.

TABLE VI

LINEAR CAPACITY OF COLUMN 2 AT VARIOUS TEMPERATURES

The solvent composition is modified each time the temperature is changed to keep the capacity factors constant.

Solute	*Parameter*	*Temperature (°C)*			
		15.7	*30*	*46.5*	*64.5*
n-Hexylbenzene	$\theta_{Max.}$*	182	195	245	363
	$\theta_{Mom.}$**	537	557	631	912
n-Octylbenzene	$\theta_{Max.}$	138	158	209	302
	$\theta_{Mom.}$	257	302	234	562
n-Nonylbenzene	$\theta_{Max.}$	141	148	234	263
	$\theta_{Mom.}$	316	288	646	661
1,2,4-Trichlorobenzene	$\theta_{Max.}$	71	67	126	73
	$\theta_{Mom.}$	103	115	148	145

* Linear capacity determined from the retention time of the peak maximum (μg/g).
** Linear capacity determined from the retention time of the mass-centre of the peak (μg/g).

We have previously reported[12] that the linear capacity is one order of magnitude smaller with pyrocarbon packings than with silica or bonded phases. Increasing the temperature does not change this result significantly. It is noteworthy that in the k'-normalized condition, there are no important differences between the capacities of pure carbon black and pyrocarbon-modified silica gel. These results may be explained in terms of lateral interactions between adsorbed sample molecules. It would seem that for highly polarizable molecules (such as trichlorobenzene) these interactions are particularly strong and begin to play an important role at lower coverage of the adsorbent surface, resulting in small values of θ. More work is needed to obtain information on this phenomenon.

Hydrodynamic and kinetic properties

Column pressure drop. Temperature has an important effect on the viscosity (η) of the solvents. This is particularly significant in reversed-phase HPLC because

the viscosity of MeOH–W mixtures can sometimes be high. Systematic measurements of the viscosity of mixed binary solvents at various temperatures and compositions were made. The results are given in Table VII for MeOH–W and MeCN–W mixtures. It is noticeable that the composition of the mixture at which the viscosity is at a maximum is independent of the temperature (55% W for the MeOH–W system and 65% W for the MeCN–W mixture). A temperature increase of 55 °C decreases the viscosity of both the MeOH and MeCN systems by a factor of 2.8.

TABLE VII

VARIATION OF THE VISCOSITY (cP) OF METHANOL–WATER AND ACETONITRILE–WATER MIXTURES WITH TEMPERATURE

The composition is given in % (v/v) of water at 20.5°C. Upper figures, methanol–water mixture; lower figures, acetonitrile–water mixture.

Temperature (°C)	*Water content (%, v/v)*										
	0	*10*	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*	*100*
15	0.63	1.05	1.40	1.69	1.91	2.02	2.00	1.92	1.72	1.43	1.10
	0.40	0.54	0.70	0.81	0.89	0.98	1.09	1.30	1.23	1.18	1.10
20	0.60	0.93	1.25	1.52	1.72	1.83	1.83	1.75	1.57	1.32	1.00
	0.37	0.50	0.56	0.69	0.81	0.90	0.99	1.13	1.10	1.14	1.00
25	0.56	0.84	1.12	1.36	1.54	1.62	1.62	1.56	1.40	1.18	0.89
	0.35	0.46	0.52	0.59	0.72	0.82	0.89	0.98	0.98	1.01	0.89
30	0.51	0.76	1.01	1.21	1.36	1.43	1.43	1.36	1.23	1.04	0.79
	0.32	0.43	0.45	0.52	0.65	0.74	0.80	0.86	0.87	0.90	0.79
35	0.46	0.69	0.91	1.09	1.21	1.26	1.24	1.19	1.07	0.92	0.70
	0.30	0.39	0.43	0.47	0.59	0.68	0.72	0.76	0.78	0.73	0.70
40	0.42	0.64	0.83	0.98	1.08	1.12	1.11	1.05	0.96	0.82	0.64
	0.27	0.36	0.41	0.44	0.54	0.62	0.65	0.68	0.70	0.72	0.64
45	0.39	0.58	0.76	0.89	0.98	1.02	1.00	0.96	0.87	0.75	0.58
	0.25	0.33	0.38	0.43	0.50	0.58	0.59	0.61	0.64	0.61	0.58
50	0.37	0.54	0.70	0.82	0.90	0.94	0.93	0.89	0.82	0.71	0.54
	0.24	0.31	0.36	0.41	0.46	0.53	0.55	0.57	0.60	0.60	0.54
55	0.36	0.50	0.65	0.76	0.84	0.88	0.88	0.84	0.77	0.67	0.51
	0.23	0.29	0.34	0.38	0.43	0.49	0.51	0.53	0.56	0.53	0.51
60	0.33	0.47	0.61	0.72	0.79	0.81	0.81	0.77	0.70	0.61	0.47
	0.22	0.27	0.31	0.35	0.41	0.46	0.49	0.50	0.53	0.52	0.47
65	0.28	0.45	0.59	0.68	0.72	0.72	0.69	0.64	0.58	0.51	0.40
	—	—	—	—	—	—	—	—	—	—	—

Increasing the column temperature will therefore result in a decrease in the pressure drop necessary to achieve a given flow-rate. However, if there is a need to normalize the capacity factor (*i.e.*, to achieve a constant analysis time and most often a constant resolution) at constant flow-rate, it will not necessarily be observed that the column pressure drop decreases with increasing temperature. Indeed, as k' decreases with increasing T, the mode of normalization leads to an increase in the

water content, which may result in a change in the viscosity of the mobile phase in either direction, depending on the starting conditions.

It may not serve any useful purpose to establish a general equation for the variation of η with T at constant k' because too many parameters have to be considered and no simple relation exists between such parameters as η and T or η and solvent composition. However, calculations were made in some particular instances as examples, and were performed as follows.

If the solute capacity factor is $k_{S_0}{}^{T_0}$, at the temperature T_0, with the solvent S_0 containing $S_0\%$ of water (eluotropic strength $\varepsilon^\circ_{S_0}$), the capacity factor in the same solvent at the temperature T will be

$$\log k'_{S_0}{}^{T} = \log k'_{S_0}{}^{T_0} + \frac{\Delta H}{2.3R}\left(\frac{1}{T} - \frac{1}{T_0}\right) \tag{9}$$

In order to obtain the capacity factor $k'^{T}_{S_1} = k'_{S_0}{}^{T_0}$, it is necessary to add water to S_0: the new solvent is S_1.

The difference between the water content of S_1 ($S_1\%$) and S_0 ($S_0\%$) is then

$$S_1\% - S_0\% = \left(\frac{\Delta H}{2.3R} \cdot \frac{T - T_0}{TT_0}\Big/a - 4.577 \cdot 10^{-3}\right) \cdot 1.90 \cdot 10^{-5}(T - 273) \tag{10}$$

Eqn. 10 shows that an important parameter determining $S_1\% - S_0\%$ is $\Delta H/a$. In the two examples chosen, $a = 765$ Å^2 (15 units) and ΔH is 2.3 kcal/mole in the first instance and 9.2 kcal/mole in the second. S_0 varies from 0 to 80%. The viscosity for solvent S_1 is obtained from Table VII. Calculations have been made at four temperatures. The results obtained are depicted in Fig. 9. The reference temperature is 15 °C. In most instances, a smaller pressure drop (or viscosity) is the consequence of a higher temperature. It is noticeable that, if S_0 is 25%, for instance, the composition of the solvent used at 65 °C to ensure the same k' will be that of the maximum viscosity. However, the effect of temperature on viscosity is so important that the maximum viscosity at 65 °C is smaller than the viscosity of any MeOH–W mixture at 15 °C.

Column efficiency. When the solvent reduced velocity ν ($= ud_p/D_m$ where u is the solvent velocity, D_m is the solute diffusion coefficient and d_p is particle size) is smaller than about 100, the reduced HETP (h = HETP/d_p) is related to ν through the equation

$$h = B/\nu + A\nu^{0.33} + C\nu \tag{11}$$

The coefficients A and B are related to the packing quality (A) and to the axial diffusion (B). Theoretically they are independent of temperature. The contribution of the mass transfer process is $C\nu$. Knox and Vasvari[1] have stated that an increase in temperature improves the column efficiency at a given solvent flow-rate, strictly because of the decrease in solvent viscosity. They particularly noted that in their systems C was independent of the temperature.

In their recent study of band broadening in LC, Horváth and Lin[20] derived

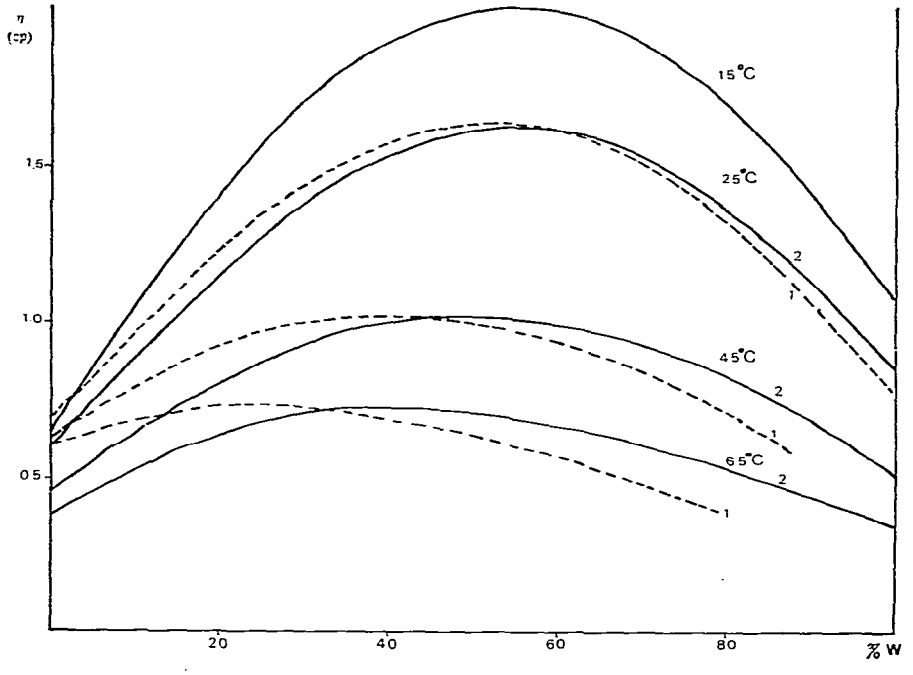

Fig. 9. Variation of the solvent viscosity with temperature and solvent composition under conditions of constant solute capacity factor. Curves 1, ΔH = 9.2 kcal/mole; curves 2, ΔH = 2.3 kcal/mole. Solvent: methanol–water mixtures; composition in % (v/v) of water.

a complete equation for the dependence between h and ν. They calculated the contribution of the kinetic processes to be given by

$$h_{\text{kin.}} = \frac{2k' D_m}{(1 + k_0)(1 + k')^2 d_p^2 k_d} \cdot \nu \tag{12}$$

where k_0 is the ratio of the interparticulate void volume to the interstitial void volume and k_d the rate constant for the solute association process with the packing. Measurements of $h_{\text{kin.}}$ at various temperatures allow the determination of the activation energy for the association process. This energy does not appear to be negligible, being of the order of −3.0 kcal/mole, and consequently temperature can have a significant effect on the mass transfer contribution to band broadening.

We have previously reported[17] that fitting experimental points to eqn. 11 could provide reliable information on the value of C only if the contribution of $C\nu$ to h is at least 10%. This indicates generally a high solvent velocity. For instance, if $C\nu$ is expected to be larger than $h/4$, it corresponds to

$$\nu \geqslant \sqrt{\frac{A^3}{27C^2}} \tag{13}$$

assuming B/ν to be negligible. With classical values for A and C (2 and $2 \cdot 10^{-2}$) this means that $\nu > 200$. On the one hand, with such reduced velocities, extra-column

contributions to band broadening may appear with small particles because of injection and detection problems[17]. On the other hand, as the maximum flow-rate of the pumping system is frequently 10 cm^3/min, the solvent velocity is limited by the particle size. For instance, with a column of only 1000 theoretical plates, a 10 cm^3/min flow-rate will generate a reduced velocity of 200 only if particles are larger than 7.2 μm. In such a case, the pressure drop in the column will be 620 bar with a solvent whose viscosity is 0.5 cP.

In the range of high reduced velocities, the variations of h with ν are well described by the simple equation

$$h = \alpha + \beta\nu \tag{14}$$

used by Endele and Halász[21]. In eqn. 14 the term $\beta\nu$ accounts for the mass transfer process in the column.

In order to cover a wide range of reduced velocities (1–5000) it appears that it is necessary to use two columns. The first, packed with small particles (20 μm, column 1) will give information on A and the second, packed with large particles (250 μm, column 8) will provide data on C (or β). This is valid only if a good packing can be achieved in both instances.

The diffusion coefficients of the solutes were calculated by the Wilke and Chang equation[22]. With the solvents used (90:10 MeCN–W for column 1 and 70:30 MeCN–W for column 8) the association coefficient of the mobile phase was assumed to be independent of the temperature. The variations of the solvents viscosities and densities were given above. The expansion coefficients for benzene, *m*-xylene and 1,2,4-trimethylbenzene are $1.182 \cdot 10^{-3}$, $0.966 \cdot 10^{-3}$ and $0.901 \cdot 10^{-3}$ °C^{-1}, respectively[23]. As we had no information for 1,2,3,4-tetramethylbenzene and pentamethylbenzene, the dilatation of these solutes was omitted in the calculations. The error is relatively small as the main contribution to the change in D_m is due to the variation in solvent viscosity. The values of D_m for the various solutes used are listed in Table VIII. Between 17 °C and 72 °C there is a 2.7-fold decrease in D_m.

(a) Small-particle column. Because of time considerations, no measurement was performed at reduced velocities of less than 1 and the coefficient B in eqn. 11 was assumed to be 2. Had this not been done, the values obtained for B from the experi-

TABLE VIII

DIFFUSION COEFFICIENTS AT VARIOUS TEMPERATURES IN THE SOLVENT MIXTURE ACETONITRILE–WATER (90:10, v/v)

Temperature (°C)	*Diffusion coefficient (cm^2/sec × 10^{-5})*				
	Benzene	*m-Xylene*	*1,2,4-Trimethylbenzene*	*1,2,3,4-Tetramethylbenzene*	*Pentamethylbenzene*
17	2.35	1.93	1.81	1.72	1.64
32.5	3.34	2.78	2.58	2.45	2.32
42.5	4.13	3.44	3.19	3.04	2.89
53	5.05	4.20	3.96	3.74	3.57
62	5.65	4.69	4.41	4.22	4.02
72	6.39	5.30	4.97	4.81	4.58

mental data would be erratic, varying between $1.7 \cdot 10^{-6}$ to 3.76. Data obtained for A and C are given in Table IX and some surves are shown in Fig. 10. Within experimental error, it appears that A is independent of the temperature and of the solute capacity factor, with an average value of about 2.2 and a standard deviation of 0.3. Lower figures given in Table IX are associated with abnormally high C values and result from an artefact of the curve fitting. A value of $A = 2.2$ means that the packing is good: more than 10,000 theoretical plates were obtained with the column used at optimum velocity. The values obtained for C are difficult to account for. In most instances C is smaller than 0.1 but the values are scattered in spite of a slight trend towards an increase in C with increasing k' and T. The experimental error is large and this reinforces our previous opinion that under such conditions ($\nu < 100$) no useful information can be derived from C values obtained by curve fitting. The curves shown in Fig. 10 illustrate this fact: although the C values are scattered, the curves are very close. Our conclusion however, parallels that given by Knox and Vasvari[1] and others: in this range of the (h, ν) curves, temperature plays no detectable role. This is true in terms of reduced HETP and velocity. However, the 2.7-fold increase in D_m between 17 °C and 72 °C results, for a given constant flow velocity, in a 2.7-fold decrease in ν, which can result, close to the minimum of the (h, ν) curves, in a 1-unit decrease in h, which means a 20–30% increase in the actual number of theoretical plates. This can sometimes be very useful, especially when columns made with particles in the 20–40-μm range are used. Alternatively, the optimum flow velocity of the column is multiplied by 2.7.

TABLE IX

COEFFICIENTS A AND C IN THE EQUATION $h = 2/\nu + A\nu^{0.33} + B\nu$

Column 1; solvent, acetonitrile–water (90:10, v/v). k' values are given at 17 °C.

Temperature (°C)	*Parameter*	*Benzene ($k' = 0.02$)*	*m-Xylene ($k' = 0.17$)*	*1,2,4-Trimethylbenzene ($k' = 0.49$)*	*1,2,3,4-Tetramethylbenzene ($k' = 1.73$)*	*Pentamethylbenzene ($k' = 4.06$)*
17	A	2.36	2.46	2.21	2.40	2.28
	C	$5.96 \cdot 10^{-8}$	$2.05 \cdot 10^{-3}$	$2.25 \cdot 10^{-2}$	$3.31 \cdot 10^{-2}$	$3.91 \cdot 10^{-2}$
32.5	A	2.21	2.33	2.20	2.30	2.43
	C	$3.96 \cdot 10^{-2}$	$4.07 \cdot 10^{-2}$	$5.18 \cdot 10^{-2}$	$6.46 \cdot 10^{-2}$	$4.72 \cdot 10^{-2}$
42.5	A	2.56	2.50	2.44	2.61	2.50
	C	$1.30 \cdot 10^{-2}$	$5.96 \cdot 10^{-8}$	$2.68 \cdot 10^{-2}$	$3.98 \cdot 10^{-2}$	$6.60 \cdot 10^{-2}$
53	A	1.20	1.72	1.87	1.98	2.02
	C	$26.53 \cdot 10^{-2}$	$15.16 \cdot 10^{-2}$	$11.29 \cdot 10^{-2}$	$13.44 \cdot 10^{-2}$	$12.82 \cdot 10^{-2}$
62	A	2.15	2.26	2.08	1.91	1.71
	C	$10.61 \cdot 10^{-2}$	$6.84 \cdot 10^{-2}$	$8.48 \cdot 10^{-2}$	$13.08 \cdot 10^{-2}$	$17.41 \cdot 10^{-2}$
72	A	1.62	1.92	1.90	1.85	2.09
	C	$13.95 \cdot 10^{-2}$	$8.50 \cdot 10^{-2}$	$8.40 \cdot 10^{-2}$	$14.19 \cdot 10^{-2}$	$12.80 \cdot 10^{-2}$

(b) Large-particle column. In order to make significant measurement of C from experimental data collected at large reduced velocities, we prepared a 230-cm long column packed with 250-μm particles. Obviously, it was not possible with this column to make HETP measurements at small reduced velocities. Typically, ν was in

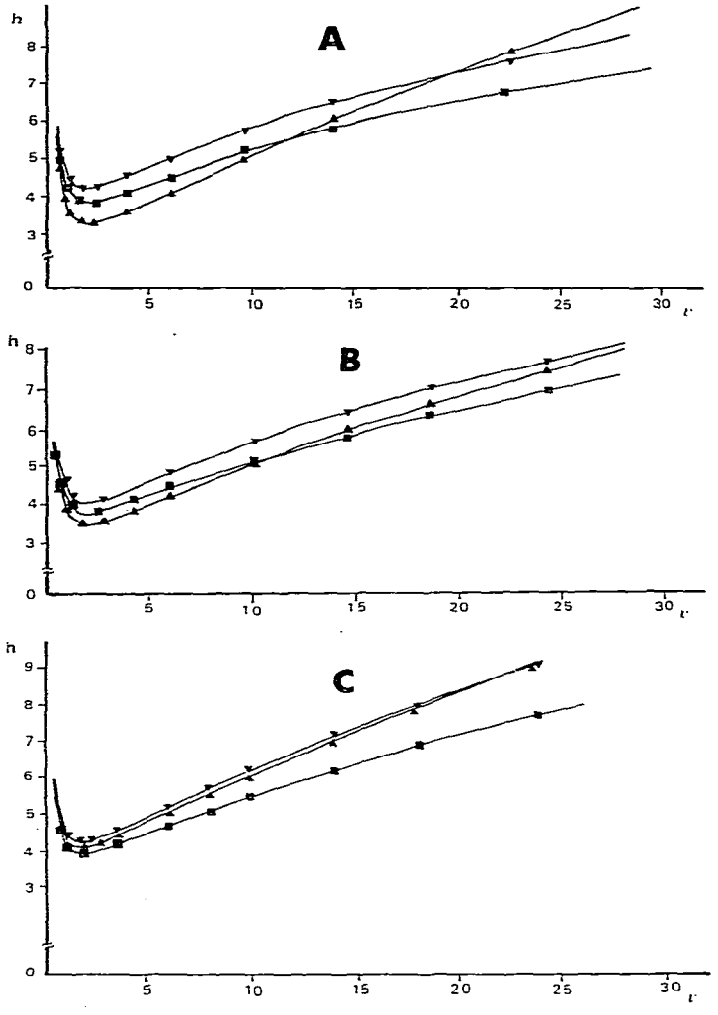

Fig. 10. Reduced HETP curves at various temperatures. Column 1; solvent, acetonitrile–10% (v/v) water. Temperature: ■, 17°C; ▼, 42°C; ▲, 72°C. (A) Benzene; (B) 1,2,4-trimethylbenzene; (C) pentamethylbenzene.

the range 250–10,000, resulting in very poor efficiencies (h between 25 and 300). The h *versus* v plots are fairly linear; the coefficients α and β in eqn. 14 are reported in Table X. It can be seen that the coefficient β (slope of the lines) decreases slowly with increasing temperature. The important drop between values obtained at 65 °C is not explained and may be accidental. The results suggest that the rate of desorption is not markedly affected by changes in the column temperature and that the major contribution to band broadening on pyrocarbon material is of thermodynamic rather than kinetic origin. This is in agreement with the results reported by Hanai and Walton[24].

Peak symmetry. Together with HETP measurements on the small-particle column, determinations of peak asymmetry have been made. We have used the same

TABLE X

COEFFICIENTS OF THE EQUATION $h = \alpha + \beta v$

Column 8; solvent, acetonitrile–30% (v/v) water.

Solute	*k' at 20°C*	*Parameter*	*Temperature (°C)* 20	40	50	65
Benzene	0.00	α	25.1	23.1	26.1	15.8
		β	$3.2 \cdot 10^{-2}$	$2.2 \cdot 10^{-2}$	$1.5 \cdot 10^{-2}$	$1.5 \cdot 10^{-2}$
1,2,4-trimethylbenzene	0.48	α	23.9	24.2	24.6	27.4
		β	$5.4 \cdot 10^{-2}$	$4.9 \cdot 10^{-2}$	$3.9 \cdot 10^{-2}$	$1.9 \cdot 10^{-2}$
1,2,3,4-tetramethylbenzene	1.41	α	39.6	29.6	26.3	44.3
		β	$6.2 \cdot 10^{-2}$	$5.7 \cdot 10^{-2}$	$5.2 \cdot 10^{-2}$	$1.8 \cdot 10^{-2}$
Pentamethylbenzene	3.14	α	20.4	30.7	53.0	91.9
		β	$8.6 \cdot 10^{-2}$	$7.9 \cdot 10^{-2}$	$5.8 \cdot 10^{-2}$	$2.7 \cdot 10^{-2}$

definition as Knox and Vasvari[1] and an example of the measurement of the asymmetry is shown in Fig. 6. The results are given in Table XI. Several conclusions can be drawn. Within experimental error, the asymmetry is independent of the flow-rate. The standard deviations (σ%) have been obtained from the results of experiments carried out at seven different flow-rates. Values of σ are between 5 and 15%, which is probably due to the experimental error made in drawing the peak inflection tangents. The asymmetry is not related to the solute capacity factor either, but rather depends on the chemical nature of the solute itself. In order to eliminate the possibility of interference between several retention mechanisms, the solutes used were polymethylbenzenes. At all temperatures, the most symmetrical peaks were obtained with 1,2,4-trimethylbenzene. The differences between the five solutes, however, are not important. Finally, increasing the temperature results in only a slight decrease in the peak

TABLE XI

PEAK ASYMMETRY (a) AT VARIOUS TEMPERATURES

Column 1; solvent, acetonitrile–10% (v/v) water. The relative standard deviation (σ%) corresponds to the results obtained at seven different flow-rates of the mobile phase. For calculation of a, see Fig. 6.

Temperature (°C)	*Parameter*	*Benzene*	*m-Xylene*	*1,2,4-Trimethylbenzene*	*1,2,3,4-Tetramethylbenzene*	*Pentamethylbenzene*
17.5	a	1.60	1.59	1.49	1.64	1.69
	σ(%)	10.4	7.5	18.0	10.2	5.6
32	a	1.58	1.55	1.51	1.60	1.72
	σ(%)	18.2	19.0	14.1	11.2	6.0
42.5	a	1.66	1.56	1.43	1.54	1.67
	σ(%)	29.6	33.9	14.7	6.9	10.4
53	a	1.41	1.36	1.21	1.48	1.56
	σ(%)	18.7	14.9	13.1	9.7	11.2
62	a	1.49	1.44	1.26	1.52	1.64
	σ(%)	18.0	10.6	24.3	13.9	9.6

asymmetry. In agreement with what has been said above, this indicates that the major contribution in determining the shape of the elution bands is of thermodynamic origin.

CONCLUSION

Temperature does not appear to be a very important parameter in reversed-phase chromatography, at least with pyrocarbon-containing adsorbents. Results obtained with pyrocarbon lead to the same conclusion as that reached by Karger *et al.*[25], who observed when working with n-C_{18} silica that there was no change in selectivity with changing temperature if the capacity factors were kept constant. Increasing the column temperature generally results in a small decrease in the selectivity on pyrocarbon because the most retained solute often has the larger enthalpy of adsorption. This heat of transfer, as affected solely by the mobile phase composition, is often larger than that reported in the literature for other reversed-phase systems. Typically, a 30 °C increase in the column temperature results in a 2-fold decrease in the capacity factor for a given solute. This effect is particularly useful with pyrocarbon materials on which adsorption is frequently strong and the retention large. The large values of the entropies of transfer denote a strong orientation of the sample molecules on the surface of the adsorbent.

The solvent eluotropic strength is not sensitive to the column temperature although, at high temperatures, the influence of the water content on the capacity factors is diminished. Results obtained in terms of the linear capacity factors, peak symmetries and column efficiencies indicate that the major contribution to band broadening is of thermodynamic rather than kinetic origin. A temperature increase of 30 °C results in only a 2-fold increase in the column capacity, the peak asymmetry remaining independent of the temperature.

The term which accounts for mass transfer in the HETP equation decreases with increasing temperature. However, under normal operating conditions, this contribution to the overall HETP is moderate or small. The major effect of the temperature is not a change in the parameters of the reduced HETP equation but the increase in the diffusion coefficients, resulting in an increase of the optimum solvent flow-rate. The pressure drop in the column is closely related to the temperature of the mobile phase. This is of practical importance for methanol or ethanol–water mixtures whose viscosity is large.

ACKNOWLEDGEMENTS

We thank Michel Margosis (F.D.A., Washington, D.C., U.S.A.) for fruitful discussions. The financial help of CNRS is acknowledged (ERA 553). We thank the French representative of Waters Assoc. for the loan of the Model 440 UV detector.

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